

1   **Patent claims**

2   1. Process for the cultivation and stimulation of three-  
3   dimensional, vital and mechanically-resistant cell  
4   transplants in a GMP-conform bioreactor with the process  
5   steps

6

7   a)   the explant cells (12) taken from the organism and  
8   prepared for bioreactor cultivation using methods which are  
9   known and the carrier matrix (13) comprising commercially-  
10   available biocompatible, absorbable or autologous or  
11   homologous materials form a cell matrix suspension after  
12   being mixed

13

14   b)   placed in a seeding piston (25) which is foiled if  
15   necessary and which has a cross-section which is adapted to  
16   the later transplant, it being hardened out or polymerized  
17   here,

18

19   c)   if necessary, has minimum pressure applied to it by  
20   means of an exactly fitting, inert stamp (26) which is  
21   structured or foiled, if necessary,

22

23   d)   the seeding piston with the transplant (11) is inserted  
24   in the chamber space of the bioreactor carcass,

25

26   e)   where the transplant (11) from the seeding piston (25)  
27   is placed medially on the floor of the bioreactor and the  
28   bioreactor (1) is closed after the seeding piston has been  
29   removed,

30

31   f)   where the transplant is subjected to further  
32   cultivation by feeding a perfusion flow into it and

33

34   g)   the replacement tissue material is removed for further  
35   use after completion of the cultivation,

36

1 characterized in that the transplant is subjected to load in  
2 the cultivation phase on the surface opposite the floor of  
3 the bioreactor.

4

5 2. Process according to Claim 1, characterized in that the  
6 transplant is loaded by a stamp which applies pressure.  
7

8 3. Process according to claims 1 und 2, characterized in  
9 that both the blending in the bioreactor chamber t due to  
10 the perfusion flow and the stamp which applies pressure to  
11 the transplant can be controlled with regard to time and  
12 quantity or density in relation to the cultivation  
13 conditions.

14

15 4. Process according to claims 1 - 3, characterized in  
16 that the transplant has conditioned cultivating medium flow  
17 through it at intervals and is subjected to loading in  
18 cycles by the stamp which applies pressure.  
19

20 5. Process according to Claims 1 - 4, characterized in  
21 that the pressure load of the transplant takes place during  
22 the perfusion.  
23

24 6. Process according to Claims 1 - 5, characterized in  
25 that the transplant is stimulated by using static,  
26 preferably in-vivo-similar pressure loads or construct  
27 deformations or they are continuously loaded by intermittent  
28 or dynamic pressure forces.  
29

30 7. Process according to Claims 1 - 6, characterized in  
31 that the mechanical load is applied with a frequency  
32 exceeding 0.1 Hz.  
33

34 8. Process according to claims 1 - 7 characterized in that  
35 the mechanical pressure stimulation is in the form of a  
36 symmetrical or asymmetrical half cosine or sine wave.

1

2 9. Process according to claims 1-8 characterized in that  
3 the pressure stamp which is from a magnetic material is  
4 moved longitudinal to the surface of the transplant in the  
5 bioreactor by an (electro-) magnetic field which is  
6 generated outside the bioreactor.

7

8 10. Process according to claims 1 - 9, characterized in  
9 that the magnetic field is generated by at least one  
10 permanent magnet.

11

12 11. Process according to claims 1-10 characterized in that  
13 at least two permanent magnets with alternating polarity are  
14 positioned on a mobile holder above the bioreactor and is  
15 driven by a servomotor in a cyclic manner, they thereby  
16 changing their position resulting in the pressure stamp  
17 applies pressure to the transplant alternately.

18

19 12. Process according to claims 1-8 characterized in that  
20 the coil of an electromagnet changes alters its current  
21 direction and voltage and therefore the field direction and  
22 magnetic flow density with high frequency via the  
23 bioreactor, resulting in the pressure stamp applying  
24 pressure to the transplant alternately.

25

26 13. Bioreactor for the cultivation and stimulation of  
27 three-dimensional, vital and mechanically resistant cell  
28 transplants in an GMP-conform bioreactor,  
29 characterized in that the bioreactor (1) of a basic carcass  
30 with a reactor lock (21). is connected in a pressure proof  
31 and sterile manner, this creating at least one reactor  
32 chamber, a storage space for a transplant (11) and a mini  
33 actuator (14) being implemented in this, the bioreactor (1)  
34 being equipped with at least two hose coupling connections  
35 (19) for the feeding and discharging of the medium in  
36 addition to the gassing.

1  
2 14. Device according to Claim 13, characterized in that the  
3 cell culture constructs (11) can be directly or indirectly  
4 cultivated and stimulated on the bioreactor floor of a  
5 single-chamber bioreactor (1).

6  
7 15. Device according to Claim 13 in that the cell culture  
8 constructs (11) are directly or indirectly, at least  
9 partially positioned on a floor of the upper reaction  
10 chamber of a double-chamber bioreactor for cultivation and  
11 stimulation, whilst this transplant (11) is in a second  
12 reactor chamber.

13  
14 16. Device according to Claims 14 or 15, characterized in  
15 that the bioreactor (1) is equipped with a transplant insert  
16 on the floor of the upper reactor chamber in which the cell  
17 constructs (11) can be placed.

18  
19 17. Device according to Claims 13 - 16, characterized in  
20 that the container of the bioreactor (1) is a cylinder-  
21 shaped corpus which is closed from above with a reactor lock  
22 (21).

23  
24 18. Device according to Claims 13 - 17, characterized in  
25 that the reactor lock unit(s) (21) and the bioreactor (1)  
26 are connected by one threaded joint and at least one conical  
27 nipple (20) in such a way that the threaded joint is either  
28 created between the reactor lock (21) and the container(1)  
29 by a female thread in the container (1) and a male thread in  
30 the reactor lock working together or the threaded joint is  
31 created between the reactor lock (21) and the container (1)  
32 in that a male thread in the container (1) and a female  
33 thread in the reactor lock (21) work together.

34  
35 19. Device according to Claims 13 - 18, characterized in  
36 that the reactor lock (21) in the form of a cover is

1 equipped with biosensors (9) and/or measuring heads (10).  
2

3 20. Device according to Claims 13 - 19, characterized in  
4 that the cover (21) is equipped with a sample taking section  
5 (10).  
6

7 21. Device according to Claims 13 - 20, characterized in  
8 that the basic carcass of the bioreactor (13) has at least  
9 two each of a feed and discharge borehole for the provision  
10 of hose coupling connections in the single-chamber  
11 bioreactor version.  
12

13 22. Device according to Claims 13 - 20, characterized in  
14 that the basic carcass of the bioreactor (1) has at least  
15 two boreholes for the provision of hose coupling connections  
16 (19) in the double-chamber bioreactor version.  
17

18 23. Device according to Claim 22, characterized in that at  
19 least one hose coupling connection (19) is integrated in the  
20 lower reaction chamber and at least one in the upper  
21 reaction chamber.  
22

23 24. Device according to Claims 21 - 23, characterized in  
24 that the feed connections (19) and discharge connections  
25 (19) which enter the bioreactor chamber are fitted with a 3-  
26 way valve (6) or a 4-way valve (7) with a return function.  
27

28 25. Device according to Claim 24, characterized in that at  
29 least one of the discharge connections (19) is provided with  
30 a sample taking section (10).  
31

32 26. Device according to Claims 13 - 25, characterized in  
33 that the bioreactor (1) has a reactor floor of a completely  
34 or partially transparent material, preferably a transparent  
35 glass plate (17) for the monitoring of the transplant  
36 manufacture.

1  
2 27. Device according to Claims 13 - 26, characterized in  
3 that a foil; fleece or membrane (18) of an antistatic or  
4 inert material is situated above the reactor floor of the  
5 bioreactor (1) for the positioning of the transplant (11),  
6 this material preferably being wide-meshed and light, fluid  
7 and gas permeable.

8  
9 28. Device according to one of the Claims 13 - 27,  
10 characterized in that when in the version of a double-  
11 chamber reactor, the upper reactor chamber of the bioreactor  
12 (1) has an area which corresponds to the transplant area  
13 whilst the dimensions of the lower chamber are under those  
14 of the transplant (11) so that if a cell culture is placed  
15 medially, this construct is mainly positioned underneath the  
16 lower chamber and is partially on the reactor floor of the  
17 upper chamber.

18  
19 29. Device according to Claim 28, characterized in that the  
20 space underneath the reactor chamber is filled out by a flat  
21 plate (16) of a biologically inert, light-permeating, wide-  
22 pored material, preferably of a porous sinter material so  
23 that this plate (16) is flush with the floor of the upper  
24 reactor chamber.

25  
26 30. Device according to Claims 28 and 29, characterized in  
27 that a foil, fleece or membrane (18) of an antistatic or  
28 inert material is situated above the lower reactor chamber  
29 which is filled out by the plate (16) on the reactor floor  
30 of the upper reactor chamber of the double-chamber  
31 bioreactor (1), this material being for the positioning of  
32 the transplant (11, it preferably being wide-meshed and  
33 permeable to light, fluid and gas.

34  
35 31. Device according to Claims 28-30, characterized in that  
36 the components which are underneath the transplant (11) in

1 the double-chamber bioreactor (1) such as the transparent  
2 plate (17), the lower chamber with the inserted porous  
3 material (16) and a wide-meshed membrane (18) have an  
4 overall height which does not exceed the focal distance of  
5 commercially available microscope and camera objectives.

6

7 32. Device according to Claim 13, characterized in that a  
8 magnetic, preferably piston-like mini actuator (14) is  
9 situated in the bioreactor (1) and can be moved through the  
10 bioreactor (1) in a controlled manner by one or more  
11 externally arranged control and steering magnets (15).

12

13 33. Device according to Claims 13-32, characterized in that  
14 the mini actuator (14) in the single-chamber bio reactor (1)  
15 is situated above the membrane (18) and the transplant (11)  
16 in the bioreactor space.

17

18 34. Device according to Claims 13-32, characterized in that  
19 the mini actuator (14) in the double-chamber bioreactor (1)  
20 is situated in the upper reactor space above the porous  
21 material (16), the membrane (18) and the transplant (11).

22

23 35. Device according to Claims 32-34, characterized in that  
24 the magnetic mini actuator (14) which is to be implemented  
25 comprises a magnetic nucleolus (22), preferably of a  
26 permanent magnet which is encapsulated in a biologically  
27 inert enveloping body (23), preferably plastic.

28

29 36. Device according to Claims 32-35, characterized in that  
30 the magnetic nucleolus (22) is so oriented that the field  
31 which is generated between the poles runs vertical to the  
32 transplant (11) so that the magnetic north pole of the  
33 complete mini actuator (14) is oriented in an upwards  
34 direction.

35

36 37. Device according to Claims 32-36, characterized in that

1 the biocompatible enveloping body (23) which surrounds the  
2 core magnets (22) has an external form which matches the  
3 form of the reactor chamber of the bioreactor (1).

4

5 38. Device according to Claims 32-35, characterized in that  
6 the complete height of the enveloping body (23) has been so  
7 selected that an implementation of the mini actuator (14) in  
8 the reactor space results in a vertically-oriented guiding  
9 of the pressure stamp of the mini actuator (14) towards the  
10 transplant (11).

11

12 39. Device according to Claims 32-38, characterized in that  
13 the piston-shaped mini actuator (14) comprises more than one  
14 enveloping body cylinder so that one of the enveloping body  
15 cylinders, preferably the upper one contains the  
16 encapsulated permanent magnet and an additional cylinder  
17 serves the stamp impression (24), whereby the spatially  
18 separated cylinders are joined by means of a bridge (34) or  
19 a connection which has the same function.

20

21 40. Device according to Claims 32-39, characterized in that  
22 the planar stamp surface (24) on the underside of the mini  
23 actuator (14) formed by the enveloping body (23, runs  
24 vertical to the guide direction in the bioreactor space (1).

25

26 41. Device according to Claims 32 and 40, characterized in  
27 that organotypical negative forms such as a convex form are  
28 embossed on the stamp surface (24) of the mini actuator  
29 (14).

30

31 42. Device according to Claims 32 and 41, characterized in  
32 that the planar or formed stamp surface (24) is embossed in  
33 the form of a grid structure in order to increase the stamp  
34 surface, this preferably having a small-meshed structure.

35

36 43. Device according to Claims 32 - 42, characterized in

1 that the enveloping body (24) of the mini actuator (14) is  
2 to be so provided with drill holes and/or flow channels (33)  
3 that a continued exact vertical guiding of the mini actuator  
4 (14) is still guaranteed at 3 points.

5

6 44. Device according to Claims 32 - 44, characterized in  
7 that the stamp surface (24) of the mini actuator (14) is  
8 fitted with at least one nosepiece which slides into its  
9 exactly fitting integrated guide rail in the bioreactor  
10 carcass.

11

12 45. Device according to Claims 32 - 44, characterized in  
13 that the control and steering magnet (15) situated outside  
14 the bioreactor (1) brings about an oriented movement of the  
15 implemented mini actuator (14) with the electro-) magnetic  
16 field which it generates with the north pole of the  
17 permanent magnet which is oriented upwards (22).

18

19 46. Device according to Claims 32 - 45 characterized in  
20 that the control and steering magnet (15) is medially  
21 situated in a vertical axis to the pressure stamp (14),  
22 preferably above the pressure stamp (14) and moves upwards  
23 and downwards in relation to the polarity of the mini  
24 actuator (14), resulting in an alteration of the pressure  
25 applied to the transplant (11).

26

27 47. Device according to Claim 32, characterized in that the  
28 control and steering magnet (15) comprises two permanent  
29 magnets (32) with different vertical magnetic pole  
30 directions which are inserted in a rectangular shaped magnet  
31 holder (31) and moved to their horizontal position above the  
32 bioreactor in a cyclic manner by means of a servomotor (29)  
33 and a guide rail (30).

34

35 48. Device according to Claim 32, characterized in that the  
36 control and steering magnet (15) comprises a minimum of two

1 permanent magnets (32) with different vertical magnetic pole  
2 directions, these being in a disk-shaped magnet holder (31)  
3 and moved over the bioreactor (1) in a cyclic manner as a  
4 result of the rotation drive of a servomotor (29).  
5

6 49. Device according to Claim 32, characterized in that the  
7 bioreactors which are firmly fixed in their horizontal  
8 position approach the permanent magnets (32) via a vertical  
9 movement of the magnet holder (31) by means of a step motor,  
10 in order to increase the field effect and generate the  
11 application of higher pressures on the transplant (11).  
12

13 50. Device according to Claim 49, characterized in that at  
14 least two bioreactors (11) are so arranged in a station that  
15 their mini actuators (14) are driven by just one permanent  
16 magnetic control system in a contactless manner.  
17

18 51. Device according to Claim 32, characterized in that the  
19 control and steering magnet (15) is in the form of an  
20 electromagnet with at least one induction coil (35) with a  
21 field which is infinitely variable using the known means.  
22

23 52. Device according to Claims 32 and 51 characterized in  
24 that die induction coil (35) is highly frequently triggered  
25 by frequencies which can be altered in order to generate  
26 high-dynamic magnetic field alterations and mini actuator  
27 (14) movement on the transplant (11).  
28

29 53. Device according to Claims 13 - 53 characterized in  
30 that a seeding piston (25) which is preferably cylindrical  
31 and has an inside diameter which corresponds to the outside  
32 diameter of the transplants on a moving sliding plate (27)  
33 for the purpose of injecting the cells (12) and the carrier  
34 matrix (13).  
35

36 54. Device according to Claims 13 - 53 characterized in

1 that the moving sliding plate (27) and the inside of the  
2 seeding piston (25) is coated by an inert membrane, foil or  
3 polymer fleece.

4

5 55. Device according to Claims 53 and 54 characterized in  
6 that an exactly fitting stamp (26) with a planar stamp  
7 surface or an organotypical negative form in the seeding  
8 piston (25) is lightly applied to the cell suspension.

9

10 56. Device according to Claims 53 - 55 characterized in  
11 that the outside diameter of the seeding piston (25) exactly  
12 matches the inside diameter of the bioreactor (1).

13

14 57. Device according to Claims 13 - 56 characterized in  
15 that at least 3 fixation walls (28) are integrated in the  
16 reactor floor of the bioreactor (1), these having dimensions  
17 which accommodate a transplant insert and do not impair the  
18 pressure compression.

19

20

21

22

23

1       List of Reference Drawings  
2

- 3   1      Bioreactor  
4   2      Medium reservoir  
5   3      Supplement reservoir  
6   4      Hose system  
7   5      Circulation pump  
8   6      3-way valve  
9   7      4-way valve  
10  8     Sample taking section  
11  9     Biosensors Glu/Lac  
12 10   Measuring heads pH, pCO<sub>2</sub>, pO<sub>2</sub>  
13 11   Transplant  
14 12   Cells/tissue  
15 13   Support matrix  
16 14   Mini actuator  
17 15   Control magnet  
18 16   Porous sinter material  
19 17   Transparent glass  
20 18   Permeable membrane  
21 19   Hose coupling/Luer connection  
22 20   Pinch ring  
23 21   Reactor lock  
24 22   Permanent magnet  
25 23   Enveloping body  
26 24   Stamp surface  
27 25   Seeding piston  
28 26   Stamp  
29 27   Sliding plate  
30 28   Fixation wall  
31 29   Servomotor  
32 30   Guide rail  
33 31   Magnet holder  
34 32   Permanent magnet  
35 33   Flow channels  
36 34   Extension nosepiece  
37 35   Coil

1    **Summary**

2  
3    Process and Bioreactor for the Cultivation and Stimulation of  
4    Three-dimensional, Vitally and Mechanically Resistant Cell-  
5    Transplants

6  
7    The task of the invention is the creation of a process and a  
8    bioreactor for the manufacturing of three-dimensional, vital  
9    and mechanically-resistant cell cultures, by which they can  
10   be cultivated and stimulated within a short time of each  
11   other or simultaneously. The bioreactor should permit GMP-  
12   conform transplant cultivation under guaranteed sterile  
13   conditions.

14  
15   The bioreactor (1) comprises a basic carcass which is  
16   connected to a reactor lock (21) so that it is pressure  
17   proof and sterile, this creating at least one reactor  
18   chamber, a storage space for a transplant (11) and a mini  
19   actuator (14) being implemented in this. The bioreactor (1)  
20   is also equipped with at least two hose coupling connections  
21   (19) for the feeding and discharging of the medium in  
22   addition to the gassing.

23  
24   The invention enables GMP-conform cultivation of three-  
25   dimensional, vital and mechanically-resistant cell cultures,  
26   preferably cartilage-cell constructs which can hereby be  
27   cultivated and stimulated in a locked mini-bioreactor  
28   simultaneously, consecutively or within a time-controlled  
29   process according to GMP guidelines. These so-called  
30   transplants which are cultivated in this manner are then  
31   available as replacement tissue material for the therapy of  
32   connective and supporting tissue defects, direct joint  
33   traumas, rheumatism and degenerative joint disease, for  
34   example and can present an alternative to the conventional  
35   (operative) therapy approaches, such as micro fracturing or  
36   drill perforation in arthrosis of the knee joint, for

1 example.

2

3 Figure 3

4

5